treated cells, a number of differentially expressed transcripts were identified and cloned. Sequence homology search revealed and matched the identified clones to 1) a small subset of genes known to be involved in angiogenesis, e.g. platelet endothelial cell adhesion molecule 1 (PECAM-1), matrix metalloproteinase 2 (MMP2), endothelin converting enzyme 1 (ECE-1), and vascular endothelial growth factor receptor 2 (VEGFR-2); 2) a large subset of known genes with known function, but not involved in angiogenesis to date; and 3) about 5-10% of the clones were novel genes with unknown function, such as a putative G-protein coupled receptor. Thus there are clusters of related gene products, differentially regulated and involved with cholinergic, proliferative and apoptotic action. cDNA microarray analysis (~48,000 elements) validated our findings Conclusion: Nicotine promotes angiogenesis through stimulation of angiogenic mechanisms partly through the cholinergic pathway. Therapeutic modulation of nAChR may be useful in disorders of angiogenesis.

9:45 a.m.

804-3 **Relation Between the $C_{677}T$ Transition in the Methylentetrahydrofolate Reductase Gene, Plasma Homocyst(e)ine and Folate Levels, and Coronary Artery Disease in the GENICA (Genetic and Environmental Factors in Coronary Atherosclerosis) Study**

Gian Paolo Rossi, Maurizio Cesari, Alberto Burlina, Stefania Colonna, Mario Zanchetta, Giuseppe Maiolino, Pietro Maiolino, Achille Cesare Pessina, Clinica Medica 4, Padova, Italy, Cittadella, Italy

Background: Hyperhomocyst(e)inemia has been implicated in atherosclerosis and can be determined by multiple environmental and genetic factors. However, the relationship of the plasma levels of homocyst(e)ine (Ho) and folate (F) with the $C^{677}T$ methylentetrahydrofolate reductase (MTHFR) gene polymorphism and coronary artery disease (CAD) has never been investigated in a large clinical dataset. **Methods**: In 964 consecutive patients (63±10 yrs, 74% men and 26% women) of the "GENICA" study, who underwent coronary angiography for suspected CAD, we measured Ho and F by HPLC and a chemiluminescence method, respectively. All were genotyped for the $C^{677}T$ MTHFR gene polymorphism by fluorescent PCR and melting curve analysis (LightCycler™). **Results:** We found a highly significant ($p<0.0001$) inverse relationship between Ho and F. A multivariate analysis identified serum creatinine, $C^{677}T$ MTHFR genotypes, F, left ventricular ejection fraction, age, and an interaction between $C^{677}T$ MTHFR genotypes and F, as significant predictors of Ho ($R^2=0.16$, $p<0.0001$). When the effect of the T MTHFR allele was examined according to a recessive model, significantly ($p<0.0001$) higher Ho values were seen in TT (14.9±0.6 µmol/l) than in CC+CT (12.0±0.3 µmol/l) patients. At variance no significant difference was seen between patients with (14.0±0.6 µmol/l) and without CAD (13.0±0.4 µmol/l). χ^2 analysis showed that high Ho (>15 µmol/l) were more common than expected in patients with history of previous myocardial infarction ($p=0.003$), peripheral vascular disease ($p<0.001$), vascular surgery ($p<0.001$) and chronic renal failure ($p<0.001$). No associations of the $C^{677}T$ MTHFR polymorphism with such outcomes were seen. **Conclusions**: These results, in patients with angiographically-assessed CAD, support the contention of Ho being determined by multiple factors, including the $C^{677}T$ transition in the MTHFR gene. Furthermore, they indicate that hyperhomocyst(e)inemia, but not the $C^{677}T$ MTHFR alleles, are associated with cardiovascular outcomes albeit not with angiographically assessed CAD.

10:00 a.m.

804-4 **Endothelin-1 Induces Expression of Functional CD40 on Human Vascular Smooth Muscle Cells**

Michael Browatzki, Caroline A. Pfeiffer, Roger Kranzhöfer, University of Heidelberg, Heidelberg, Germany

Background: Chronic inflammation of the vessel wall is a hallmark of atherosclerosis. This inflammatory process is maintained by a variety of cytokines generated in the vessel wall. Recently, activation of vascular cells by cell-cell contact via the CD40/CD154 system has been identified as important pathway of inflammatory stimulation in atherogenesis. Human vascular smooth muscle cells (SMC) as important cellular component of the atherosclerotic plaque can express both cytokines and the CD40/CD154 system. On the other hand, the vasoactve peptide endothelin-1 (ET-1) is supposed to contribute to atherogenesis. This study investigated whether ET-1 stimulates the inflammatory response in SMC via a CD40/CD154 dependent pathway. Methods and Results: ET-1 (10 nM max) like the positive stimulus interferon-gamma (100 U/ml) increased CD40 mRNA and protein expression after 24 hours in human SMC. This ET-1 effect was mediated by the ET-A-receptor subtype since BQ-123, a selective ET-A receptor antagonist, prevented ET-1-induced CD40 upregulation whereas BQ-788, an ET-B-receptor antagonist, did not (10 µM each). ET-1 also activated the proinflammatory transcription factors NF-kappaB and AP-1 in a time dependent manner. The specific proteasome inhibitor PI-1 (50 µM) and a NF-kappaB decoy oligodesoxynucleotide prevented ET-1-induced CD40 expression demonstrating dependence of this ET-1 effect on NF-kappaB activation. To test the functional relevance of the CD40 expression, SMC were preincubated with 10 nM ET-1 for 24 hours and afterwards stimulated with recombinant CD154 (5 ng/ml). Release of interleukin-6 (IL-6) into the culture medium was assessed by ELISA. Cells preincubated with ET-1 secreted a significantly higher amount of IL-6 under CD154 stimulation than control cells (265 +/- 4 vs 147 +/- 8 pg/ml, $p < 0.05$). Conclusion: ET-1 induces an inflammatory response in human SMC via direct cell-cell contact which is mediated by the CD40/CD154 system. This mechanism may contribute to the pathogenesis of atherosclerosis.

10:15 a.m.

804-5 **Decreased Caveolin-1 Expression in Atheroma: Loss of Antiproliferative Control of Vascular Smooth Muscle Cells in Human Atherosclerosis**

Carsten Schwencke, Alexander Schmeisser, Rolf Wachter, Brigitta Weck, Rainer Marquetant, Michael Kasper, Ruth H. Strasser, University of Technology Dresden, Dresden, Germany

Background: Proliferation of vascular smooth muscle cells (VSMC) is involved in the pathogenesis of primary atherosclerosis and restenosis after angioplasty. On the background of the recently proposed antiproliferative activities of caveolin-1 the present study investigated the expression of caveolin-1 in proliferating VSMC in vitro and especially in human atheroma.
Methods and Results: Primary VSMC express high levels of caveolin-1 as shown by immunoblotting. Supplementation of serum or growth factors such as PDGF caused a decrease in caveolin-1 expression in VSMC. Cell-cycle entry was documented by a decrease of the Cdk inhibitor p27kip1 and an increase of the proliferating cell nuclear antigen (PCNA). We further investigated the expression of caveolin-1 in VSMC of human atheroma using immunohistochemistry. In contrast to control vessels, caveolin-1 was markedly decreased in sections derived from human atheroma. The proliferation of VSMC in atheroma was confirmed by an increased PCNA immunostaining.
Conclusion: This newly characterized decreased expression of caveolin-1 both in proliferating smooth muscle cells in vitro and in human atheroma in vivo strongly links the loss of the antiproliferative control by caveolin-1 to the development of atherosclerosis, suggesting a pivotal role of caveolin-1 in the pathogenesis of atherosclerosis.

ORAL CONTRIBUTIONS

811 Pulmonary Hypertension and Pulmonary Embolism: Clinical Insights

Monday, March 31, 2003, 11:00 a.m.-12:15 p.m.
McCormick Place, Room S102

11:00 a.m.

811-1 **Pulmonary Artery Systolic Pressure in Echocardiographically Normal Subjects**

Richard V. Milani, Carl J. Lavie, Yvonne E. Gilliland, Krishnamoorthy Vivekananthan, Mark M. Cassidy, Jose Alberto Bernal, Ali Morshedi, Ochsner Clinic Foundation, New Orleans, LA

Backgrond: Pulmonary hypertension (PHTN) has undergone renewed interest of late with the increasing prevalence of obesity and the reported association of various anorectic agents and its subsequent effect on the pulmonary vasculature. Recent enhancements in echocardiographic instrumentation, has refined the detection of small degrees of tricuspid regurgitation in subjects, and mild "elevations" of pulmonary artery systolic pressure (PASP) are now a common finding, resulting in concern as to whether this represents true pathology. Previous definitions of PHTN suggested that PASP exceeding 30 mmHg were pathologic, however this data was often derived from small numbers of relatively young patients.
Methods: We have analyzed PASP from our echocardiographic database of 35,815 subjects, resulting in 2,472 subjects (mean age 54.9 +/- 16.3 years) which met the echo criteria of normal hearts, defined as: normal left and right ventricular dimension and function; normal left and right atrial dimensions; absence of aortic root dilatation or pericardial disease; absence of valvular stenosis; absence of valvular insufficiency less than moderate.
Results: The mean PASP was 33.1 +/- 7.7 mmHg, and correlations were found with age ($p=0.0001$), male gender ($p=0.0025$), septal wall thickness ($p=0.0001$) and posterior wall thickness ($p=0.0001$). 60% of this population had a PASP > 30mmHg, and 22% of those older than 50 years, and 20% of those with BMI > 30 kg/m2, had a PASP > 40mmHg.
Conclusions: Elevations of PASP is relatively common in echocardiographically normal populations and correlate to age and BMI. Previous definitions of normal PASP should be revised to adjust for age.

11:15 a.m.

811-2 **Inhibition of Phosphodiesterase-5 and Nitric Oxide Similarly Reduce Pulmonary Artery Pressure at High Altitude**

Hans P. Brunner-La Rocca, Patrick Egger, Oliver Senn, Manuel Fischler, Rahel Thalmann, Konrad Bloch, Marco Maggiorini, University Hospital, Zurich, Switzerland, University Hospital, Basel, Switzerland

Background: At high altitude, hypoxia induces pulmonary hypertension which plays an important role in the pathophysiology of high-altitude pulmonary edema. Inhaled nitric oxide (NO) was shown to reduce pulmonary artery pressure (PAP), but it is not applicable in parctice. Therefore, we investigated the effects of the PDE5-inhibitor sildenafil (Sil) on PAP at high altitude in comparison with inhaled NO.
Methods: Doppler-echocardiography was performed in 22 healthy mountaineers (10 W, 12 M, age 29±12y; O_2-saturation 75±3%) 3 hours after they reached an altitude of 4559m. Measurements were repeated after NO (40ppm), Sil (50mg), and Sil plus NO. PAP was estimated from tricuspid regurgitation and pulmonary vascular resistance

(PVR) from a previously described formula using preejection time, acceleration time and total systolic time. Right atrial pressure (RAP) was estimated from respiratory change of the vena cava inferior.

Results: see table. *p<0.05 vs baseline, †p<0.05 vs NO / Sil. On the next morning, measurements returned to baseline values.

Conclusion: Inhibition of PDE-5 reduces pulmonary hypertension to a similar extent as NO. As expected, the 2 agents have synergistic effects. Thus, PDE-5 inhibitors may be candidates for prevention of high-altitude pulmonary edema in susceptible mountaineers.

	Baseline	NO	Sil	Sil+NO	Sig.
PAP	44±10	32±6*	33±6*	28±5*†	<0.0001
PVR	84±20	72±27	67±22*	64±27*	0.002
RAP	5.9±1.3	5.3±1.4	4.7±1.1*	3.8±1.3*	0.03

11:30 a.m.

811-3 Prognostic Value of Transthoracic Echocardiography in Massive Pulmonary Embolism: A Retrospective Study of 617 Patients

Gérard D. Pacouret, E. Hamel, M. Montout, D. Djeffal Vincentelli, B. Charbonnier, Trousseau Hospital, Tours, France

Right ventricular dilation induced by massive pulmonary embolism (PE) can be easily detected, at bedside, by trans-thoracic echocardiography (EC). To test the prognostic value of this parameter, we retrospectively analyzed a population of 617 consecutive patients admitted to our institution for acute massive PE, and who were evaluated by EC at baseline. Massive PE was defined as angiographic Miller index ≥ 20/34 and/or perfusion lung scan defect ≥ 40 % and/or bilateral central PE on helicoidal CT scan.

Results : Right ventricle/left ventricle end diastolic diameter ratio (RV/LV), calculated from the long axis parasternal view, the subcostal view or the apical 4 chamber view in supine position was assessable in 548 patients (89 %). 27 pts died during the index hospitalization (4.4 %). Univariate analysis revealed that among 7 parameters, only systemic hypotension and RV/LV were significantly associated with the risk of death (see table).

After adjustment to systemic hypotension, contra-indication to thrombolysis and thrombolysis, RV/LV appeared as an independent predictor of in-hospital death (p=0.008) in multivariate analysis.

Conclusion : RV/LV end-diastolic diameter ratio is an independent predictor of in-hospital death in patients suffering from acute massive PE. Thrombolysis could be beneficial in such patients when RV/LV > 0.8, even in the absence of hypotension. A prospective randomized study is mandatory to confirm this hypothesis.

Univariate Analysis Of In-Hospital Risk Factors

	Death	Alive	p
	n=27	n=590	
Age (95% CI)	73.4 (68.4-78.4)	71.4 (70.4-72.4)	0.41
Gender (M/F) (n)	8/19	239/351	0.31
Previous Thrombo-E mbolism (n)	10	219	> 0.99
Contra-Indication to Thrombolysis (n)	14	228	0.23
Thrombolysis (n)	11	169	0.20
RV/LV (95% CI)	1.00 (0.82-1.17)	0.79 (0.77-0.81)	< 0.0001
Systemic Hypotension (n)	12	101	0.001

11:45 a.m.

811-4 Effect of Treprostinil Sodium on Circadian Regulation of Heart Rate Variability in Primary Pulmonary Hypertension

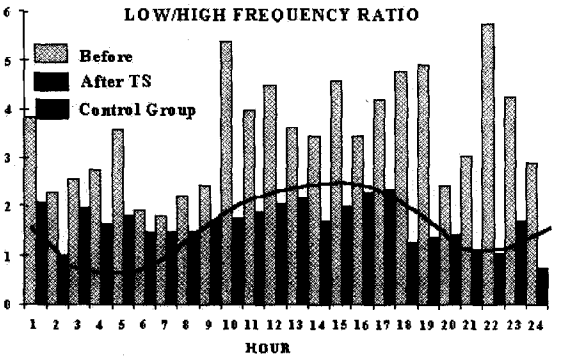
Martín P. Rosas, Julio Sandoval, Nuria Granados, Fause Attie, Tomás Pulido, Teresa Miranda, Efrén Santos, Instituto Nacional de Cardiologia Ignacio Chavez, Mexico City, Mexico

Background: A reduction of heart rate variability (HRV) is now considered as an independent risk factor for morbidity, mortality and severity of several cardiac diseases; however, the dynamic sympathovagal modulation on HRV during 24 hr in primary pulmonary hypertension (PPH) and the therapeutic impact of Treprostinil Sodium (TS) on this modulation has not been described.

Methods: 24 hr-Holter monitoring (HM) were recorded in 15 patients (mean age 34 ± 12; 90% female) with severe PPH (mean pulmonary pressure = 70 ± 12 mm Hg), before and after three months of therapy with subcutaneous TS. The HRV time and spectral parameters (mean, SDNN, SDANN, rMSSD, PNN50, LF, HF and LF/HF ratio) were analyzed during three periods: 24 hr; Day (8-22:00), night (23-07:00) and also during every hour of recording (5 min-intervals).

Results: Circadian rhythm of HRV is clearly disturbed in PPH (increased sympathetic tone) as compared to normal control subjects (p < 0.05). Frequency parameters of HRV during 24 hr-HM were significantly different before and after long-term subcutaneous TS administration (Figure).

Conclusions: The circadian rhythm of HRV in PPH is lost mainly due to an increase of sympathetic tone. This autonomic imbalance may favor the development of arrhythmia, heart failure, and sudden death. The recovery of circadian regulation of HRV detected after long-term TS may be mediated by an improvement in central hemodynamics as a result of TS therapy.



Noon

811-5 Long-Term Survival Comparison in Patients With Primary and Secondary Pulmonary Arterial Hypertension Treated With Chronic Epoprostenol Therapy

Maninder S. Bedi, Jessica Spates-Panyon, Michael M. Mathier, Ajoy Kapoor, Guy A. MacGowan, Dennis M. McNamara, Srinivas Murali, University of Pittsburgh, Pittsburgh, PA

Pulmonary arterial hypertension (PH), whether primary (P) or secondary (S) due to systemic sclerosis (SS) has a poor prognosis. Epoprostenol (E) therapy improves symptoms and survival in PH, though it is not known if the survival benefit from E is different in PPH and SPH patients (pts).

Methods: Baseline hemodynamics (prior to E therapy) and outcomes were assessed in 92 PH pts (Grp A: PPH, n=61, age 44.3± 11.8 years, 87% female, NYHA Class III/ IV 57/ 28%; Grp B: SPH, n=31, age 52.8± 11 years, 74% female, NYHA Class III/ IV 45/ 32%) treated with E infusion. Both Grps had normal baseline resting left ventricular ejection fraction (Grp A: 57±5%, Grp B: 55±5 %). Doses of E were comparable (Grp A: 56±33, Grp B: 57±37 ng/kg/min) and pts were followed for 809± 625 days (range 8-3256 days).

Results: At baseline, Grp A had significantly higher (p<0.05) pulmonary artery (PA) diastolic (38.6±10.9/ 32.8±7.6mmHg), PA Mean (56.4±13.7/49.9±8.8 mmHg) pressures compared to Grp B. Right Atrial and PA systolic pressures, Cardiac index and pulmonary vascular resistance were comparable in the Grps. During follow-up, 16(26%) Grp A and 3(10%) Grp B pts underwent pulmonary transplantation and 19(31%) Grp A and 11(36%) Grp B pts died. The one, two and five year transplant free survival (figure) in the two Grps was comparable (Grp A: 82/59/35%; Grp B: 89/67/38%, p=0.4).

Conclusion: Long-term survival on E therapy is similar in PPH and SPH due to SS, despite higher PA pressures in PPH pts at initial presentation.